Sarcoptes mite from collection to DNA extraction: the lost realm of the neglected parasite

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REVIEW

Sarcoptes mite from collection to DNA extraction: the lost realm of the neglected parasite

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Abstract *Sarcoptes* mite from collection to DNA extraction forms the cornerstone for studies on *Sarcoptes scabiei*. Whilst the new science era took a shy leap into the different facets of mite studies, the cornerstone was almost entirely neglected. Mite collection, cleaning, storage and DNA extraction were, basically, humble attempts to extrapolate, adapt, modify or 'pirate' those existing methods to the peculiarities of *Sarcoptes* research. These aspects usually constituted few lines, bashfully mentioned, in the materials and methods section of some papers, which arose in unique problems concerning cost-effectiveness, time profitability, safety and even worse, the credibility of the results, creating contradictory conclusions in some cases. This 'noisy' situation encouraged us to collect, classify and review, for the first time to our knowledge, some aspects relating to studies on *Sarcoptes* mite from collection to DNA extraction, which will be useful for further studies on *Sarcoptes*, and have implications for the effective control of the diseases *Sarcoptes* mite causes. Further studies are needed, especially to compare the profitability, safety, sensibility and specificity of the different methods of this neglected realm of the ubiquitous ectoparasite.

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Introduction

Worldwide, *Sarcoptes scabiei* causes mange in companion, livestock and wild mammals and scabies in humans. It has a very broad host range, including more than 100 mammalian species belonging to 27 families and ten orders (Bornstein et al. 2001; Pence and Ueckermann 2002). Scabies is the first disease of man with a known cause, and its causative agent *Sarcoptes* mite was described and illustrated by the Italians Bonomo and Cestoni in 1689 (Montesu et al. 1991).

However, whilst recent advances are providing new insights into scabies mite biology and genetic mechanisms (see Mounsey et al. 2008, for a review), genetic studies on *S. scabiei* have been extremely limited. This is primarily due to the difficulty in obtaining sufficient numbers of individual mites (in ordinary scabies, less than ten organisms can be identified per host; Mellanby 1944) and adequate amounts of genetic material (because of the high rate of failed reactions; Walton et al. 1997; Cruickshank

2002; Berrilli et al. 2002; Alasaad et al. 2008c). Moreover, many molecular techniques are not universally applicable to mites because of the general lack of DNA sequence and genetic information (Weeks et al. 2000).

The epidemiology, morphology, pathology, diagnosis, management, control, treatment, host–parasite interaction and genetics of *S. scabiei* have been discussed, in detail, in recent reviews (Burgess 1999; Navajas and Fenton 2000; Bornstein et al. 2001; Pence and Ueckermann 2002; McCarthy et al. 2004; Walton et al. 2004b; Heukelbach and Feldmeier 2006; Hengge et al. 2006; Walton and Currie 2007). The purpose of this review article is not to duplicate or repeat, but rather to supplement and complement those reviews, where *Sarcoptes* mite collection, cleaning, storage, disruption and genomic DNA (gDNA) extraction have not been discussed properly (Table 1). Such knowledge will be useful to facilitate *Sarcoptes* mite diagnosis and morphological examination and to encourage the wider use of mite experimental infestations, in vitro bioassays and molecular studies.

Studies on profitability, safety, sensibility and specificity of the different methods of *Sarcoptes* mite from collection to DNA extraction were limited in mite literature (Alasaad et al. 2009). Under these circumstances, the criteria that we used to compare and classify the different methods were mainly based on the authors' personal experience.

Mite collection

Aqueous potassium hydroxide digestion technique

This technique is in large use to recover *Sarcoptes* mites from live and dead hosts since the direct microscopic examination of scrapings or pieces of the mangy skin is often not worth the effort.

The first application was described by Hopkins (1949), and then it was modified by Cook (1954). The basic technique consists of skin digestion by trypsin and the dissolution of the skin and the hair by KOH. This recovery technique was the subject of many modifications and improvements during the last years. The aim of those modifications/improvements was to optimise skin digestion by the incubation at different temperatures for several hours and to improve mite observation and counting condition by mite concentration, using centrifugation method, flotation method or a combination of both (Table 2). Using one or both concentration methods at the same time is more efficient than the direct examination of the KOH solution (Alonso et al. 1998). Heating is not required and even resulted in a poorer visibility of the morphological features (Zahler et al. 1999).

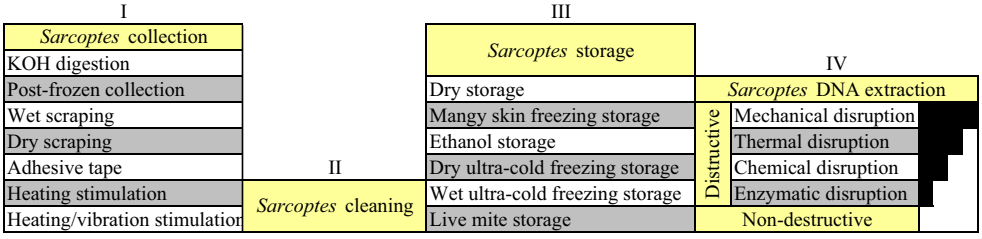
The purpose of the collected mites, in this case, is for *Sarcoptes* diagnosis (which includes the determination of the intensity of mite infection) and/or mite morphological examination since no mite biological quality is required for this purpose. Although this technique is characterised by high sensibility, mites collected by KOH digestion are not fit for experimental infestation, in vitro bioassays or DNA extraction purposes (Table 3).

Postponed (post-frozen) collection technique

Alasaad et al. (2008a, d; 2009) reported that frozen skin ('postponed isolation' method) is suitable source for mite diagnosis purpose and/or for the extraction of PCR-quality gDNA, where the mangy skins were conserved at −20°C for long time. This could be an alternative method for *Sarcoptes* mite collection since mite collection for these purposes is usually reported from fresh skins, using somewhat labour-intensive and time-consuming techniques (e.g. Brimer et al. 1993; Smets and Vercruysse 2000; Shanks et al. 2000), with their associated risk of field personnel infestation (Menzano et al. 2004).

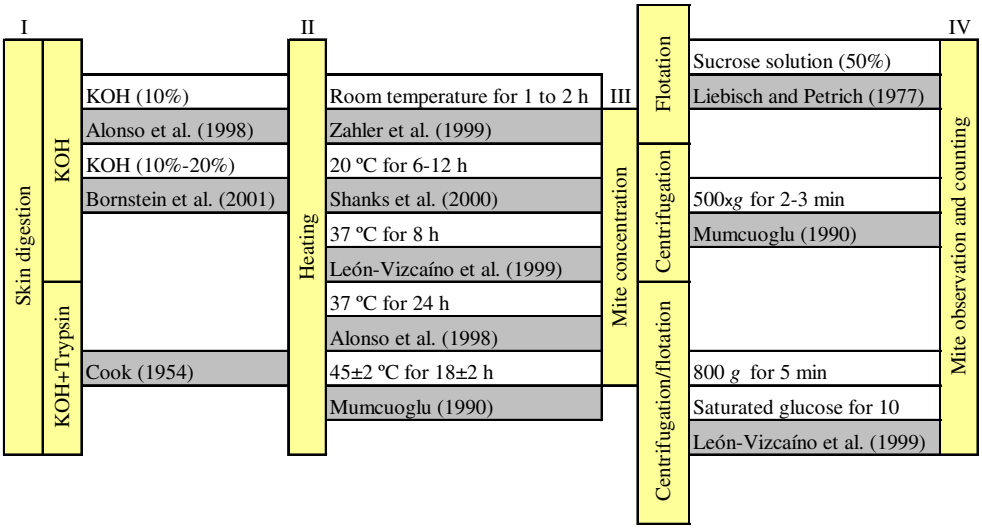
Post-frozen isolation is a suitable and recommendable method for *Sarcoptes* mite diagnosis and gDNA preparation, particularly when performing a balancing act among safety, practicability and profitability.

Table 1 Step-by-step *Sarcoptes* mite from collection to DNA extraction



The coordination between the different steps from collection to DNA extraction is represented by the size of boxes. The black bars represent the frequency of mite DNA extraction methods in the literature

Table 2 Step-by-step *Sarcoptes* mite recovery technique based on KOH digestion



Wet scraping technique

Wet scarping can be made with the following: (1) mineral oil—One or two drops of mineral oil are applied to the lesion, which is then scraped or shaved. The collection step is followed by mite transfer to a microscope slide under a coverslip and mite examination using a dissecting microscope within 24 h (Leibowitz 1994; Walton et al. 1999a). (2) Distilled water—The scraping is made by a scalpel or similar bladelike tool on Petri plates, and this step is followed by dilution of crusts in tap water and collection of dead mites with a needle under a dissecting microscope (Alasaad et al. 2008c).

Dry scraping technique

In this case, skin scrapings are made with a scalpel or similar bladelike tools to the point of oozing blood on the living or dead host, then samples are transferred to a microscope slide under a coverslip and viewed using a dissecting microscope within 24 h (Walton et al. 1999a). Fthenakis et al. (2000) described, in detail, the dry skin scraping for live animals as follows: Skin scrapings from three lesion sites bordering healthy tissue were obtained from each animal, and approximately 1 cm^2 at each site was scraped. A scalpel blade was dipped into glycerine; a skin fold was pinched between the forefinger and the thumb and, whilst holding the blade at right angle to the skin, scraped until blood seeped from the abrasion. Samples were examined within 6 h of collection.

Wet and dry scraping could be rapid methods for mite diagnosis and collection, but the sensibility and specificity should be lower than the other mite collection methods, taking into account that (1) no total digestion of the mangy skin is done and that (2) these methods have a lack of mite concentration (centrifugation/vibration), comparing to HOK digestion method. The final purpose of these two techniques is mite diagnosis and/or morphological examination. As well, they could be used for *Sarcoptes* mite DNA extraction since no high biological quality is required for mite DNA extraction (Walton et al. 1999a; Zahler et al. 1999).

Table 3 *Sarcoptes* mite collection methods, together with the mite final study purposes

Sarcoptes collection	Safety	Bio. quality	*Sarcoptes* final study purpose			Examples
KOH digestion						Bornstein et al. 2001
Post-frozen collection						Alasaad et al. 2009
Wet scraping						Alasaad et al. 2008c
Dry scraping						Fthenakis et al. 2000
Adhesive tape				DNA extraction	Diagnosis and /or morph. examination	Katsumata and Katsumata 2006
Heating stimulation			Exp. infestiation and/or in vitro bioassays			Shanks et al. 2000
Heating/vibration stimulation						Sheahan and Hatch 1975

The black bars represent the safety of the collection methods for the handling personnel and the quality of the collected mites for the biological studies

Adhesive tape technique

The standard method of diagnosis for scabies by wet/dry skin scraping requires some skill. Therefore, Katsumata and Katsumata (2006) employed the adhesive tape method to catch the mites, using a strong transparent adhesive tape commercially available for packing use, as an alternative method for the diagnosis of scabies infestation. After firmly applying the adhesive side of the tape onto an appropriate skin lesion of patients, the tape was pulled off and transferred directly onto a slide for microscopy, affixing the adhered separated part of the corneal skin.

With adhesive tape technique, we could observe some mites moving around and also see a striated structure with dark red or weak red colour or grey colour granule-like constitution on the body of some mites. This technique should be useful for mite DNA extraction. Notwithstanding, this technique is not recommended for epidemiological studies on *Sarcoptes* mite since it serves for mite diagnosis only in cases of severe mite infestations.

Heating stimulation technique

Heating stimulation techniques are successful in recovering *Sarcoptes* mite from live or dead hosts. On the living host, deep skin scrapings are made with a scalpel or similar bladelike tool to the point of oozing blood, whilst in the case of dead hosts, pieces of mangy skin are removed. Skin scrapings or pieces of mangy skin are placed in a Petri dish. Heating from the light source of a stereomicroscope (for ~1 h) stimulates mites to migrate from the skin. Mobile mites are separated under a stereomicroscope (Bornstein et al. 2001). The heating temperature for this technique ranged between 28°C and 37°C, and the incubation time reported was 10 min (Shanks et al. 2000), 30 min (Walton et al. 1999a; Smets and Vercruysse 2000) or 1–3 h (Brimer et al. 1993; Bornstein et al. 2001; Brimer et al. 2004). Pérez (2002) reported small modifications of this technique, where small pieces of mangy skins from naturally infected Iberian ibexes were collected into Petri dishes, and the dishes were placed on black background (except a small transparent central circle). Heating from the light source of a stereomicroscope (for ~18 h) focussed on the lower part of the dishes stimulated mites to migrate from the skin pieces to the transparent central circle because of the resulting temperature gradient.

Heating and vibration stimulation techniques

The heating source in these techniques is a magnetic stirrer hot plate. Skin of mangy animals and parakeratotic crust are placed in Petri dishes in a metal tray, which is vibrated and warmed by a magnetic stirrer hot plate. This stimulates mites to move out of skin and parakeratotic crust. Then, Petri dishes are examined with a stereomicroscope (Sheahan and Hatch 1975; Skerratt 2001; Skerratt et al. 2002).

Both methods (heating and heating/vibration stimulation) are suitable for mite diagnosis, morphological examination, DNA extraction, experimental infestation and/or in vitro bioassays since the collected *Sarcoptes* mites are live and with high biological quality. Nevertheless, these methods should not be used for mite diagnosis or for the epidemiological studies on *Sarcoptes* since we can collect only live stimulated *Sarcoptes* mite, and this is not a criterion for mite diagnosis and is not representative of mite intensity in the mangy skin, or at least no study was done to estimate such relation between the real mite population size in the mangy skin and the live collected mites. Another handicap of the heating/vibration stimulation techniques is that these techniques are relatively labour intensive, time consuming and may arise risk for field personnel infestation (Alasaad et al. 2009).

Mite cleaning

The aim of the so-called mite cleaning is to remove, partially, the external micro-flora of the live mites collected with live mite recovery techniques. It was applied by Brimer et al. (2004) on *S. scabiei* var. *suis* as follows: Scrapings from the inside of the ear of infected slaughterhouse pigs were transferred to empty polystyrene Petri dishes, which were subsequently incubated at 35°C for 1–2 h. Mobile mites were separated under a stereomicroscope and transferred to Petri dishes containing solidified sterile agar (Columbia agar base 40 g/l and 5% *v*/*v* horse serum). The agar plates with the mites were incubated at 35°C for 30 min.

Mite cleaning is a useful practice for mite infestation and/or in vitro bioassays. It could be applied before mite DNA extraction (Table 1).

Mite storage

Dry storage

This mite storage method could be useful for morphological studies, but drying is not generally good for DNA extraction, and therefore mites collected from museum specimens may not be suitable for molecular work (Cruickshank 2002).

Freezing storage of mangy skin

See "Postponed (post-frozen) collection technique" section.

Ethanol storage

The universally used storage method for diagnosis and/or morphological examination of mites is to fix *Sarcoptes* samples in 70% alcohol (e.g. Walton et al. 1999a; Berrilli et al. 2002). Ethanol storage is also widely used for *Sarcoptes* DNA extraction purpose since the other storage methods, which guarantee mites with high biological quality (dry/wet ultra-cold freezing and live storage), are not always practicable. An acceptable alternative is mite storage in 100% ethanol (at −20°C if possible) as soon as possible. At lower concentrations of ethanol, the DNA is rapidly degraded, giving variable results, and hence, it should be avoided if at all possible (Weeks et al. 2000). However, mites stored in ethanol as low as 70% for many years can give good yields of DNA (Walton et al. 1999a; Berrilli et al. 2002). Using acetone as an alternative to ethanol is also possible (Fukatsu 1999).

Dry ultra-cold freezing storage

Evidence from insects suggests that the most effective method for preserving specimens for molecular work is ultra-cold freezing (−80°C) of live specimens (Post et al. 1993; Reiss et al. 1995; Dillon et al. 1996). Walton et al. (1999a) used the dry ultra-cold freezing storage at −20°C. Weeks et al. (2000), Skerratt (2001) and Skerratt et al. (2002) stored mites at −70°C, whilst Walton et al. (2004a) stored at −80°C.

Wet ultra-cold freezing storage in digestion buffer

In this method, *Sarcoptes* mite samples are placed into digestion buffer and then stored at −80°C. Walton et al. (1997) used 50 mM Tris–HC1, 1 mM EDTA and 0.5% sodium dodecyl sulphate (SDS; pH 8.5) as digestion buffer and stored at −70°C. Mounsey et al. (2005) used 500 mg/ml proteinase K, 50 mM Tris–HCl, 1 mM EDTA and 0.5% SDS (pH 8.5) as digestion buffer and stored at −80°C.

Dry and wet ultra-cold freezing storage methods guarantee a high quality of the stored mites for DNA extraction purpose. Drawbacks of these techniques derive from the life history of *Sarcoptes* samples, where opportunistic ones are often collected in remote areas (Dagleish et al. 2007), and hence the practicable use of the other mite storage methods, like the freezing storage of mangy skin.

Live mite storage

This storage technique is usually used for mite experimental infestation and/or in vitro bioassays since the time that *S. scabiei* can live out of the host (time-off) is so limited for this kind of experiments. Detailed studies by Arlian et al. (1984) demonstrated that human and canine scabies mites are capable of surviving for 24–36 h at room conditions (21°C and 40–80% relative humidity) and still remain infective and capable of penetration. However, the time required for complete penetration of the epidermis increased as a function of time-off the host, attesting to the mite's weakened condition. Therefore, live storage for this purpose (experimental infestation and/or in vitro bioassays) will be quite short, to circumvent differences in survival ability. Brimer et al. (1993, 1995) mentioned the possibility of storage of scrapings to be used for harvesting of live mites in closed vials at 4°C for 2–3 days. Walton et al. (2000) reported the examination of the mites, utilised for in vitro bioassay, within 3 h of collection. Skerratt (2001) informed that mites were either kept at room temperature or kept at 10°C overnight before being transferred to experimental wombats.

Ideally, it would be preferable to directly utilise *Sarcoptes* mite samples for their final study purpose. Nevertheless, such 'idealism' is not always available, or at least not workable, with mite 'investigation reality'. An acceptable alternative is the storage of the collected mite samples. More or less, mite time storage depends on the final purpose of the collected parasites and on the required mite biological quality. On the other hand, mite time storage has big repercussions on the effectiveness and profitability of the mite studies (Table 4). For instance, the quality of mite DNA depends on the storage conditions of specimens prior to extraction and subsequent extraction protocol (Weeks et al. 2000). Moreover, the ratio of mite PCR failed reactions is highly influenced by the quality of mite storage methods. Walton et al. (1999b) reported more than 50% of failed PCR reactions, supposedly, related to the

Table 4 *Sarcoptes* mite storage methods, together with the time and temperature of storage

Sarcoptes storage	Storage temperature	Storage time	Bio. quality	Time-off	Examples
Dry storage	Room temperature	undetermined			Cruickshank 2002
Mangy skin freezing storage	From -20˚C to -80˚C	Years			Alasaad et al. 2008a
Ethanol storage	Room temperature	Years			Berrilli et al. 2002
Dry ultra-cold freezing storage	From -20˚C to -80˚C	Years			Walton et al. 2004a
Wet ultra-cold freezing storage	From -20˚C to -80˚C	Years			Mounsey et al. 2005
Live mite storage	From 4˚C to 10ºC	Up to 2-3 days			Brimer et al. 1993

The black bars represent the quality of the collected mites for the biological studies and mite time-off the host

delay in transporting the skin scraping. Alassad et al. (2008d) informed of 39% failed reactions; hence, the severe effect of mite storage method on the profitability of mite DNA extraction, taking into account the low parasite charge of *Sarcoptes* in the ordinary scabies (less than ten organisms can be identified per host; Mellanby 1944), and therefore the required high rate of successful reactions.

Mite disruption and DNA extraction

Numerous publications described the preparation of gDNA from mammalian and invertebrate tissues (e.g. Henry et al. 1990; Cockburn and Fritz 1996; Truett et al. 2000). These methods, often designed for cultured cells and tissues, generally require some form of cell lysis followed by deproteination procedures and subsequent DNA recovery (Sambrook et al. 1989). Nevertheless, the nature of *S. scabiei*, namely the small size (adult females measure 300–500×230–340 μm and adult males 213–285×160–210 μm wide; Fain 1978) and the difficulty in disrupting (because of the hard chitinous exoskeleton), prevented the direct application of DNA extraction methods, designed for invertebrate tissues, on *Sarcoptes* mite genetic studies. The playing key roles of the disruption methods, on mite DNA preparation, formed the background of our following classification of mite DNA extraction methods. Nevertheless, the literature on *Sarcoptes* mite DNA preparation is swarming with combinations of different mite disruption methods (Table 1).

Destructive mite DNA extraction

Mite DNA extraction based on enzymatic disruption

Proteinase K digestion is a common alternative for rapid preparation of un-purified PCR-quality DNA from many tissues (Truett 2006). Originally, proteinase K was employed to remove histones and other proteins, which bound so tightly to DNA that they resisted extraction with phenol alone (Hilz et al. 1975). Published methods for the extraction of DNA from the Acari rely on the use of proteinase K and SDS extraction buffers in conjunction with phenol extraction and DNA concentration by ethanol precipitation (Dobson and Barker 1999; Passos et al. 1999). Nevertheless, there were no reports using only proteinase K, without any disruption method, to extract *Sarcoptes* mite gDNA because of the chitinous exoskeleton of this hard-bodied ectoparasite. Proteinase K has been usually used with the combination of other mite disruption techniques (Walton et al. 1997; Zahler et al. 1999; Berrilli et al. 2002; Mounsey et al. 2005), which will be discussed in the following.

Mite DNA extraction based on chemical disruption

Walton et al. (1999b) used the chemical disruption technique described by Beige et al. (1995) for DNA extraction from *Mycobacterium tuberculosis* to extract mite gDNA: Pre-treatment with 2.5% *N*-acetyl-L-cysteine–NaOH solution was applied, then the cell pellets were lysed with 100 μl of 50 mM NaOH for 15 min at 95°C under an oil overlay, then material was neutralized with 1 M Tris–HCl (8 μl per 50 μl of NaOH). Mite DNA extraction based on chemical disruption also was reported by Weeks et al. (2000) as follows: Individual or pooled mites were collected and crushed in 100 μl of CTAB buffer (100 mM Tris, 20 mM EDTA, 1.42 M NaCl, 2% PVP-40 and 2% CTAB), vortexed for 10 s, incubated at 65°C for 15 min and vortexed again for 10 s. This was followed by adding 1 volume of chloroform/*iso*-amyl-alcohol (24:1), mixing the solution slowly and centrifuging at 12,000 rpm for 15 min at room temperature. The supernatant was then transferred to a sterile 500 μl test tube with 2 volumes of ice-cold 100% ethanol and 1/10 volume of NaAc (3 M) added and subsequently stored at −20°C for 30 min. After incubation, the test tube was centrifuged at 12,000 rpm for 20 min at 4°C. After centrifugation, the supernatant was removed, leaving behind the pellet of DNA, which was then vacuum-dried for 10 min and dissolved in 20–40 μl of sterile water.

Mite DNA extraction based on thermal disruption

The protagonist factor in these disruption methods is the change of temperature. Alasaad et al. (2008b) reported a new technique for preparing PCR-quality *Sarcoptes* mite gDNA, namely HotSHOT Plus ThermalSHOCK. This technique was based on some modifications of the HotSHOT technique, which has been widely used on different tissue types (Truett et al. 2000). The sodium hydroxide (25 μl), the first reagent, was applied as a substrate for three cycles of thermal shock (2 min at −80°C, freezing step, and 15 s atc +70°C, thawing step), followed by a short incubation (95°C for 30 min) and pH adjustment with a 25 μl Tris solution (HotSHOT Plus ThermalSHOCK). No difference in performance was observed between HotSHOT Plus ThermalSHOCK and the traditional DNA preparation using proteinase K digestion, concluding that DNA prepared by HotSHOT Plus ThermalSHOCK is as good as the DNA prepared by the traditional method. No effect was observed of heating time on the yield of soluble *Sarcoptes* mite DNA prepared by HotSHOT Plus ThermalSHOCK technique, with the recommendation of heating time between 20 and 60 min. This technique was time saving, economic and easily automatable for the preparation of PCR-quality mite DNA.

Mite DNA extraction based on mechanical disruption

In these methods, the basic rule was the snap freezing of mite samples, followed by the mechanical disruption using a mortar, needlepoint, pestle, micro-fuge tissue grinder or a motorised micro-pestle. Walton et al. (1997) used a micro-fuge tissue grinder to crush the mites used for preparation of DNA for cloning. Mites were stored previously into digestion buffer at −70°C. Firstly, a high-speed homogenisation with a micro-fuge tissue grinder was required (Kontes, cat. no. 749540). Fifty to 100 mites per micro-fuge tube were re-suspended in 0.5 ml of GES (5 M guanidinium thiocyanate, 100 mM EDTA and 0.5% sarkosyl). The suspensions were homogenised with the micro-fuge tissue grinder for 5 min and 0.25 ml of 7.5 M ammonium acetate added. After extraction with chloroform/*iso*-amyl alcohol (24:1) and ethanol precipitation, the DNA was re-suspended in TE (1 mM Tris–HC1, 0.1 mM EDTA, pH 8.0). Zahler et al. (1999) used the mechanical disruption method to extract the DNA from isolated mites or skin scraping (less than 100 mg), not single mites, as follows: Skin scrap or isolated mites were frozen in liquid nitrogen and ground to a fine powder in a mortar. The powder was re-suspended in 400 μl TET buffer (50 mM Tris–HCl (pH 8.0), 25 mM EDTA, 100 mM NaCl, 0.4% Triton X-100) and 5 μl (20 mg/ml) proteinase K. After incubation at 50°C for 2 h, the DNA was extracted by shaking with 400 μl PCI (phenol/chloroform/*iso*-amyl alcohol=25:24:1), which had been saturated with 10 mM Tris–HCl (pH 8.0) containing 1 mM EDTA. After centrifugation, the DNA was purified from the aqueous phase by silica gel adsorption (Geneclean; Bio 101) and eluted in 120 μl distilled water. Skerratt (2001) and Skerratt et al. (2002) reported slight modifications of this method, where DNA was extracted from mites by adding 10 μl of water to the tubes, centrifuging the tubes to ensure mites were at the bottom, briefly placing the tube into liquid nitrogen to freeze the mite and water, and then crushing the mite and ice with a pestle. Then, 100 μl of water containing 5% chelex beads (Biorad) was added to each tube. DNA was eluted by heating the chelex solution to 97°C for 10 min. Berrilli et al. (2002) placed individual mite samples into a 1.5-ml micro-tube and kept them in liquid nitrogen for a few seconds to facilitate the rupture of the cell membranes. The tissue was crushed by a pestle in 100 μl Grind buffer (1% SDS/25 mM NaCl/25 mM EDTA) and lysed with 12 μl proteinase K (20 mg/ml). After incubation at 56°C for 2 h, DNA was purified with one phenol–chloroform and one chloroform extraction and two ethanol precipitations. The pellet was re-suspended in 50 μl distilled water. Mounsey et al. (2005) used motorised micro-pestle to crush mite samples: Frozen mites were homogenised in either 20 or 50 μl of PrepMan Ultra solution (Applied Biosystems, Foster City, CA, USA) using a motorised micro-pestle (Kontes). The samples were then boiled for 10 min and cooled on ice.

Mechanical disruption methods seem to be highly effective as numerous applications of this disruption method have been reported in the literature of *Sarcoptes* genetic studies.

From all mite DNA extraction methods, described above, we would tend to the favour of DNA extraction methods described by Beige et al. (1995; chemical disruption) and Alasaad et al. (2008b; thermal disruption), as they are single-tube methods in which the tube is entered only twice, to add the mite samples for the lysis procedure and to withdraw samples for the amplification procedure. These simple lysis procedures are especially important for controlling the risk of cross-contamination between specimens. In addition, the lower risk of contamination with the one tube alkaline preparation methods also increase the sensitivity of the PCR system because there is no loss of specific DNA as seen in all other DNA preparation methods that need multiple pipetting steps and changing of tubes.

Klompen (2000) found it necessary to use two to five mite individuals in each extraction, whilst Anderson and Trueman (2000) used only leg tissue dissected from individual mites. In the case of *Sarcoptes* mite, it is advisable to extract DNA from single individuals, not from pooled mite samples, to prevent mixing of distinct genotypes, particularly if there is any question about the identity of the mites; otherwise, we may have contradictory conclusions. For instance, Zahler et al. (1999) did not detect clear-cut evidence of genetic separation relating to host species or geographic origin by analysing 23 pooled samples of mite isolates, whereas Berrilli et al. (2002), using the same DNA marker with DNA isolated from individual mites, described a similar degree of genetic polymorphism but with clear-cut evidence of genetic separation relating to host species and geographic origin. The difference in the results obtained may be related to the fact that the former study was based on pooled samples, and genetic polymorphism among single individuals may have been underestimated. Hence, it is important to extract DNA from individual mites, but not from pooled mites, in order to detect the possible existence of intra-population polymorphisms.

Non-destructive mite DNA extraction

When extracting DNA, it is usual to slide mount a few individuals as voucher specimens so that they are available for examination subsequent to DNA sequencing (Ruedas et al. 2000). Ideally, the voucher specimen should be the individual that is sequenced but this individual is usually completely destroyed during the extraction process (Rose et

al. 1994; Phillips and Simon 1995). Cruickshank et al. (2001) have adapted the DNeasy Tissue Kit from Qiagen (http://www.qiagen.com/) for non-destructive DNA extraction of lice by increasing the duration of the lysis step. This method has also been applied to midges (*Culicoides* sp.), and specimens have been successfully identified to species after DNA has been extracted. This method has also been tested on mites, and complete grinding of the specimen was only found necessary for the very small mites, although there was some loss of yield in larger mites (Cruickshank 2002). Further studies are needed to apply the non-destructive method on *Sarcoptes* mite studies.

Conclusion

Only recently, attempts have been made to develop techniques for the early identification of scabies infections and for molecular studies on *Sarcoptes* mites. Severe technical limitations, regarding to availability of sufficient numbers of *Sarcoptes* mites and adequate amounts of genetic material which underutilised molecular studies on *Sarcoptes* mite, have been overcome by several approaches during the last years. Nevertheless, there is still much to be done and more to be understood in the epidemiology, morphology, pathology, diagnosis, management, control, treatment, host-parasite interaction and genetics of *Sarcoptes* mite, not to mention human scabies diagnosis, which unfortunately is the daily duty in many sanitary centres, with about 300 million cases worldwide (WHO 2001).

This article reviews and classifies literatures regarding sample collection, cleaning, storage, disruption and DNA extraction of this cosmopolitan parasite, which we hope would serve as a practical and useful guide.

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